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Fatty acid-binding proteins in the diagnosis and disease severity prediction in pneumonia

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Abstract
Aim: Infections of the respiratory tract are important healthcare problems that are one of the main causes of referrals to the emergency department. PSI and CURB-65 are the most common scoring methods globally with proven accuracy and validity through many studies. Fatty acid- binding proteins are member of small cytoplasmic proteins that play a role in the transportation and deposition of lipids almost in all mammalian cells. They are strongly associated with metabolic and inflammatory processes. The aim of the present study was to determine the value of FABP for diagnosis and disease severity in patients diagnosed with pneumonia and to compare the correlation with PSI and CURB-65 scoring systems.
Material and Methods: This prospective and single-cantered study was conducted on patients referring to the emergency department of Istanbul Kanuni Sultan Suleyman Training and Research Hospital who were diagnosed with pneumonia and on healthy volunteers.
Results: FABP level was significantly higher in the patient group when compared with the control group (p: <0.01). FABP level was detected significantly higher in the severe pneumonia group of the binary groups created according to PSI and CURB-65 scoring.
Discussion: As a result of the data obtained in the present study, it was concluded that FABP would be useful for the determination of the diagnosis, disease severity and the decision whether to hospitalize the patient with pneumonia. FABP is an important biomarker that guides the clinician for management of pneumonia patients who refer to the emergency department.

Keywords
Pneumonia, Inflammation, Infection, Biomarker, FABP

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Introduction

Respiratory infections, one of the main reasons for presenting to the emergency department, constitute an important health problem. The occurrence of lung infections without recent exposure to hospitals or healthcare units is defined as community-acquired pneumonia (CAP) and considered an important cause of morbidity and mortality. Despite advancements in medical science, CAP-related mortality remains to be the same over the past four decades in the US [1]. Each year over 5 million cases occur in the USA with over 1 million hospitalizations and 60,000 deaths from pneumonia [2]. CAP is the second most common cause of hospitalization and the most common cause of death associated with infectious diseases in the US [3]. It is well-established that the mortality rate increases in the presence of comorbid disorders.

Fatty acid-binding proteins (FABP), which are a part of the cytoplasmic protein group, are strongly associated with metabolic and inflammatory processes. In macrophages, FABPs induce an inflammatory effect via the nuclear factor-κB pathway [4,5]. The present study aimed to investigate the importance of FABP in diagnosing pneumonia and predicting disease severity in patients presenting at an emergency department of a third-line education and research hospital and determine the correlation between PSI and CURB-65 scoring systems.

Material and Methods

Formation of Study Groups

This was a prospective single-center study that was conducted after obtaining approval from the relevant ethics committee of the third-line training and research hospital. For this study, all patients diagnosed with pneumonia in the emergency room were included in the patient group. Age, gender, vital signs, Glasgow Coma Scale scores, medical history, hemogram results, biochemistry and blood gas parameters, PSI and CURB-65 scores, and FABP levels of the patients were recorded.

The patients categorized under Class I and II based on their PSI scores had a low rate of mortality; thus, outpatient treatment was recommended. Accordingly, the patients categorized under PSI Class I and II were included in the PSI mild-pneumonia subgroup, whereas those categorized under PSI Class III, IV, and V were associated with high mortality rates and were recommended to get admitted to the general ward or intensive care unit were included in the PSI severe-pneumonia subgroup. With regard to the CURB-65 scoring system, the patients with a score of 0 and 1, having low mortality rates, and who were recommended to undergo outpatient treatment were included in the CURB-65 mild-pneumonia subgroup, whereas those with a score of ≥2 were included in the CURB-65 severe-pneumonia subgroup.

The patient group included individuals aged ≥ 18 years who were diagnosed with pneumonia in the emergency department, consented to participate, and had no other acute causative factor that could have affected the FABP level.

The control group included individuals aged ≥ 18 years who had no known chronic diseases or signs of active infection, did not have any recent occurrence of (up to 1 week) respiratory infection, and consented to participate.

Exclusion Criteria

Patients aged < 18 years and those who were pregnant, refused to provide consents, and had any causative factor that might affect the FABP level were excluded from the study.

Laboratory Methods

Biochemical analysis

Venous blood samples were collected from the patient and control groups via routine phlebotomy and transferred into 5-ml gel tubes (BD vacutainer SST II Advance, NJ, USA) and 2-ml anticoagulant tubes (K2-EDTA, Becton Dickinson, NJ, USA). Furthermore, arterial blood samples were collected from the patient group and transferred into 2-ml blood gas tubes (Sarstedt Monovette, 2-ml LH) for use during PSI scoring. The samples in the blood gas and anticoagulant tubes were immediately examined. The samples in the gel tubes were used to perform biochemical examinations for scoring, and the remaining samples were stored at room temperature for 20 min and then centrifuged at 3500 rpm for 10 min to obtain serum and plasma samples. At the same time, excess serum and plasma samples were transferred into Eppendorf tubes and immediately frozen at −80oC for further use in enzyme-linked immunosorbent assay (ELISA).

ELISA

The amount of human FABPs was examined in all the samples using the sandwich ELISA method. The Synergy HTX BioTek device (Biotek Instruments, Inc Highland Park, USA), antibodies against human FABP, and ELISA kit of the Bioassay Technology Laboratory company were used. The intra- and interassay coefficients of variation of the kit were <8% and 10%, respectively.

Statistical Analyses

In this study, statistical analyses were performed by first comparing the main groups, i.e., the control and patient groups, and then the PSI and CURB-65 subgroups. Data were analyzed using the Statistical Package for the Social Sciences Version 26.0 (IBM Inc., New York, USA) software program. Mean ± standard deviation and median [interquartile range (IQR)] values were used for representing continuous variables, whereas categorical variables were represented as numbers (percentages). The Mann–Whitney U test was used for performing binary group comparisons of the continuous variables without normal distribution, whereas the Kruskal–Wallis test was used to compare three or more groups and Pearson's chi-square test was used to compare categorical data. Since the most informative biomarker was FABP, its cutoff level was calculated, and the sensitivity, specificity, and positive and negative predictive values of PSI and CURB-65 were determined and compared. A p-value of <0.05 was considered statistically significant.

Results

As per pairwise comparisons that investigated patient outcomes from the emergency room and FABP levels of the patient group, the median FABP level was 25.135 ng/ml (IQR: 23.477–31.092 ng/ml) in the outpatient group, 29.692 ng/ml (IQR: 28.926–30.999 ng/ml) in the patients admitted to the ward, and 60.14 ng/ml (IQR: 49.592–106.857 ng/ml) in the patients admitted to the intensive care unit. A significant

Table 1. Intergroup comparison of blood parameters and demographic characteristics

Parameter	Control Group	Patient Group	p-value
Number of men (%)	19 (43,4)	25 (%55,6)	0,393[b]
Number of women (%)	22 (53,6)	20 (44,4)	
Mean age ± SD	63,44±13,71	65,87±14,41	0,427[a]
Leukocyte count (×103/μL)	7,55 [6,35-9,37]	11,2 [9,06-17,81]	<0.01[c*]
Hematocrit (%)	39 [35,70-42,75]	34,7 [29,15-44,55]	0,072[c]
Platelet count (×103/μL)	238 [204-281]	239 [183-309]	0,839[c]
Neutrophil count (×103/μL)	4,34 [3,66-5,14]	9,76 [7,34-14,94]	<0.01[c*]
Lymphocyte count (×103/μL)	2,40 [1,70-2,80]	1,10 [0,65-1,70]	<0.01[c*]
NLO	2,01 [1,30-2,91]	9,02 [5,43-15,05]	<0.01[c*]
PLO	103,85 [78,28-134,24]	221,54 [132,18-319,64]	<0.01[c*]
CRP (mg/L), mean ± SD	1,66±1,07	159,21±114,65	<0.01[a*]
FABP (ng/ml)	26,467[21,01-32,22]	35,896 [26,80-58,13]	<0.01[c*]

NLO: Neutrophil/lymphocyte ratio, PLO: Platelet/lymphocyte ratio, CRP: C-reactive protein, FABP: Fatty acid-binding protein. Variables with normal distribution are presented as mean ± standard deviation and variables without normal distribution are presented as median [interquartile range]. [a]Independent t-test, [b]Pearson's chi-square test, [c]Mann–Whitney U test, *p < 0.05

difference was found between the FABP levels of the patients admitted to the intensive care unit and those of the patients admitted to the ward and outpatient group (p < 0.01, p < 0.01, respectively). There was no significant difference between the patients admitted to the ward and the outpatients in terms of FABP levels. Nevertheless, the FABP levels of the patients admitted to the ward were significantly high (p = 0.056) (Table 2).

For differentiating mild and severe pneumonia, the receiver operating characteristic (ROC) curve analysis was used to determine the FABP cutoff level, which helped determine whether inpatient or outpatient treatment should be provided on the basis of PSI scores (Figure 1). Furthermore, ROC curve analysis was used to determine the cutoff level after a significant difference was found between the patient and control groups in terms of plasma FABP levels (p < 0.01) (Figure 1). When 40.333 ng/ml was set as the cutoff plasma FABP level, its sensitivity, specificity and positive and negative predictive values for diagnosis was found to be 44.44%, 97.56%, 95.24%, and 61.54%, respectively.

Table 2. Relationship between age and laboratory parameters and PSI and CURB-65 scores of the patient group

Parameter	CURB-65 mild (n = 20)	CURB-65 severe (n = 25)	p-value	PSI mild (n = 8)	PSI severe (n = 37)	p-value
Age	59[47,5-65]	71[66-76]	<0,01*	49[40,5-62]	69[63-76]	<0,01*
Leukocyte count (×103/μL)	10,65[9,06-17,48]	12,49[9,14-17,15]	0,64	12,69[9,2-17,93]	11,2[9,12-15,42]	0,929
Hematocrit (%)	32,25[27,45-41,5]	36,4[30,1-45,2]	0,283	36,9[29,1-40,5]	33,1[29,2-45,1]	0,767
Platelet count (×103/μL)	255,5[186,5-315]	205[180-239]	0,161	244,5[178-459,5]	239[186-307]	0,678
Neutrophil count (×103/μL)	9,17[7,48-14,48]	11,01[7,54-14,52]	0,648	10,95[7,62-15,13]	9,76[7,54-13,26]	1
Lymphocyte count (×103/μL)	1,25[0,60-1,65]	1[0,8-2,1]	0,963	1,45[0,55-2,25]	1,1[0,7-1,4]	0,634
NLO	12,64[5,61-14,68]	7,87[5,18-15,22]	0,615	9,41[5,31-14,14]	9,02[5,48-15,22]	0,733
PLO	247,42[154,27-309,64]	213,64[123,85-320]	0,631	207,71[91,67-329,33]	221,54[136,67-319,29]	0,614
PSI score	82[70-95,5]	114[99-143]	<0,01*	65[50,5-69,5]	106[85-126]	<0,01*
FABP level (ng/ml)	29,103[23,961-53,642]	42,132[30,312-60,248]	0,038*	26,508[23,961-37,770]	38,921[28,926-60,248]	0,035*
CRP level (mg/L)	133[69,43-224,49]	143,29[57,71-212,12]	0,802	135[63,1-210,9]	143,3[65,2-212,1]	0,882

NLO: Neutrophil/lymphocyte ratio, PLO: Platelet/lymphocyte ratio, CRP: C-reactive protein, FABP: Fatty acid-binding protein, Median [interquartile range], Mann–Whitney U Test, *p < 0.05

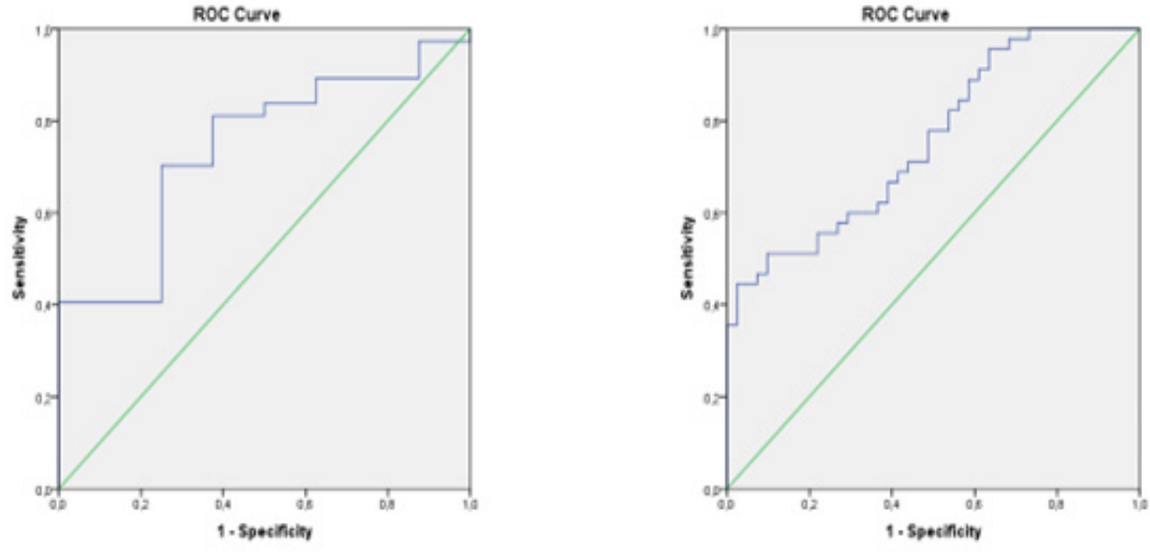

Figure 1. ROC curve comparing FABP levels for determining whether inpatient or outpatient treatment should be provided based on PSI scores and the ROC curve comparing FABP levels between the patient and control groups for the diagnosis of CAP

When 30.002 ng/ml was set as the cutoff plasma FABP level, the sensitivity of plasma FABP level was 70.27%, specificity was 75%, positive predictive value was 92.86%, and negative predictive value was 35.29% for differentiating mild and severe pneumonia using PSI scores.

Discussion

Despite advancements in medical science, antimicrobial agents, and supportive treatment options, pneumonia remains to be an important cause of mortality and morbidity worldwide. While mortality rates associated with many infectious diseases have decreased owing to the aforementioned developments, there has been no decrease in the rate of mortality in patients with CAP over the past four decades.

In a study by Wang et al. that included 36 pediatric patients and 28 controls, the plasma FABP levels were significantly higher in the patient group than in the control group [6]. In the present study, a comparison between the control and patient groups suggested that FABP levels were significantly high in the patient group, which was considered valuable for diagnosis ($p < 0.01$). In animal studies, the production of cytokines, such as tumor necrosis factor-α, interleukin-1 and -6, and monocyte chemotactic protein-1, was found to be suppressed in the absence of FABP. It was also observed that the production and functioning of proinflammatory enzymes, such as inducible nitric oxide synthase and cyclooxygenase were suppressed [4,5]. These data suggest the fact that FABP could be used as a diagnostic biomarker in patients with pneumonia.

In their prospective observational study published in 2019, Ham et al. compared the ability of PSI and CURB-65 scoring systems and various laboratory parameters in determining the severity of CAP. Leukocyte and platelet counts, C-reactive protein (CRP), and procalcitonin test (PCT) levels did not reveal any significant difference between the two scoring systems [7]. In a study by Menendez et al., CRP levels were significantly different between the two scoring system subgroups [8]. In a study by Thiem et al., which retrospectively examined 391 advanced-age CAP cases in Germany, leukocyte counts and CRP levels were not associated with the subgroups of the scoring systems [9]. A meta-analysis published in 2020 by Ebell et al. compared leukocyte count, CRP levels, and PCT for the diagnosis of CAP. It was suggested that the most accurate results were obtained using the CRP biomarker [10]. These findings suggest that leukocyte count, CRP, and PCT can successfully diagnose CAP and predict survival. Nevertheless, this is not the case when it comes to determining the provision of inpatient or outpatient treatment.

Conclusion

FABP levels were significantly higher in the CURB-65 and PSI severe-pneumonia subgroups (for which inpatient treatment was recommended) than in the CURB-65 and PSI mild-pneumonia subgroups (for which outpatient treatment was recommended) (p: 0.038, p: 0.035). These data suggest that FABP levels can be used as an objective scale in correlation with the scoring systems for determining the provision of inpatient or outpatient treatment. Despite the fact that no severity marker blood parameters in the laboratory findings were suggested in the relevant literature, in the present study, the FABP levels of the patients admitted to the intensive care unit were significantly higher than those of the outpatients and patients admitted to the ward ($p < 0.01$, $p < 0.01$, respectively). A relatively small group of evaluated patients and the fact that long-term mortality rate of the patients was not included in the study data are the limitations of the study.

When compared with PSI and CURB-65 scoring systems, the FABP levels were found to correlate with both the scoring systems in terms of predicting patient outcome. FABP may guide clinicians in diagnosing pneumonia, assessing disease severity, and predicting patient outcome.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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